PHAGE.001DV1 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Kordyum, et al.	) Group Art Unit Unk
Appl. No.	:	Unk	)
Filed	:	Herewith	)
For	:	**PHAGE-DEPENDENT SUPER PRODUCTION OF BIOLOGICALLY ACTIVE PROTEIN AND PEPTIDES**	)
Examiner	:	Unk	)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the application as follows:

IN THE SPECIFICATION:

Please insert the following before the first line of the specification:---This application is a divisional application of U.S. Serial Number 09/318,288, filed May 25, 1999.---

IN THE CLAIMS:

Please cancel claims 1-40.

Please add the following new claims:

41. (New) A method for producing a biologically active protein, comprising:

transforming an *E. coli* host cell with a plasmid having at least one copy of an expressible eukaryotic gene encoding said protein;

lytically infecting the transformed bacterial host cell with a bacteriophage λ having cI_{857}, Q_{am117}, and R_{am54} mutations; and

cultivating the *E. coli* host cell under a culture condition that induces lytic growth of said cell without lysis until a desired level of production of said protein is reached, wherein said protein is produced as a soluble, biologically-active protein.

42. (New) The method of claim 41, wherein the protein is human alpha-2b.

43. (New) The method of claim 41, wherein the host cell further comprises recA$^-$13.

44. (New) The method of claim 41, wherein the *E. coli* host cell produces a suppressor for the repair of amber-mutations.

45. (New) The method of claim 41, wherein the *E. coli* host cell lacks a suppressor for the repair of amber-mutations.

46. (New) The method of claim 41, wherein the infecting bacteriophage λ is provided at a multiplicity of infection in a range of about 1 to about 100.

47. (New) The method of claim 41, wherein the infecting bacteriophage λ is provided at a multiplicity of infection in a range of about 10 to about 25.

48. (New) The method of claim 41, wherein lysis of the *E. coli* host cell is delayed at higher multiplicities of infection relative to lower multiplicities of infection.

49. (New) A method for producing a biologically active protein, comprising:

transforming an *E. coli* host cell with a plasmid having at least one copy of an expressible eukaryotic gene encoding said protein;

lytically infecting the transformed bacterial host cell with a bacteriophage λ having cI_{857}, Q_{am117}, and R_{am54} mutations, wherein the bacteriophage also contains at least one copy of said expressible gene encoding said protein; and

cultivating the *E. coli* host cell under a culture condition that induces lytic growth of said cell without lysis until a desired level of production of said protein is reached, wherein said protein is produced as a soluble, biologically-active protein .

50. (New) The method of claim 49, wherein the strain of *E. coli* produces a suppressor for repairing amber-mutations.

51. (New) The method of claim 49, wherein the strain of *E. coli* lacks a suppressor for repairing amber-mutations.

52. (New) The method of claim 49, wherein said protein is human alpha-2b interferon.

53. (New) A method for producing a biologically active protein, comprising:

transforming an *E. coli* host cell with a plasmid having at least one copy of an expressible eukaryotic gene encoding said protein;

lytically infecting the transformed bacterial host cell with a bacteriophage λ having at least one mutated gene selected from the group consisting of N, Q, and R;

providing conditions to delay lysis; and

cultivating the *E. coli* host cell under a culture condition that induces lytic growth of said cell without lysis until a desired level of production of said protein is reached.

54. (New) The method of claim 53, wherein the bacteriophage λ has a temperature-sensitive mutation.

55. (New) The method of claim 54, wherein the temperature–sensitive mutation is cI_{857}.

56. (New) The method of Claim 53, wherein said strain of *E. coli* lacks a suppressor for repairing amber-mutations.

57. (New) The method of Claim 53, wherein said strain of *E. coli* is recA deficient.

58. (New) A method for producing a biologically active protein, comprising:

transforming an *E. coli* host cell with a plasmid having at least one copy of an expressible eukaryotic gene encoding said protein;

lytically infecting the transformed bacterial host cell with a bacteriophage λ, having at least one mutated gene selected from the group consisting of N, Q, and R, wherein the bacteriophage also contains at least one copy of said expressible gene encoding said protein;

providing conditions to delay lysis; and

cultivating the *E. coli* host cell under a culture condition that induces lytic growth of said cell without lysis until a desired level of production of said protein is reached.

59. (New) The method of claim 58, wherein the bacteriophage λ has a temperature-sensitive mutation.

60. (New) The method of claim 59, wherein the temperature–sensitive mutation is cI_{857}.

61. (New) The method of Claim 58, wherein said *E. coli* host cell lacks a suppressor for repairing amber-mutations.

62. (New) The method of Claim 58, wherein said *E. coli* host cell is recA deficient.

63. (New) A method of producing a biologically active protein comprising:

growing a first strain of *E. coli* cells, which harbor a strain of bacteriophage λ, wherein the bacteriophage λ has a temperature-sensitive mutation,

manipulating the temperature to provide for lysis of the first strain of *E. coli* cells and release of the bacteriophage λ,

lytically infecting a second strain of *E. coli* cells with the released bacteriophage λ, wherein said second strain of *E. coli* cells has been transformed with a plasmid having at least one copy of an expressible gene encoding said protein; and

culturing the second strain of *E. coli* host cells such that protein is produced and released to the media, wherein said protein is produced as a soluble, biologically-active protein.

64. (New) The method of claim 63, wherein the temperature–sensitive mutation is cI_{857}.

REMARKS

Claims 1-40 have been cancelled. Claims 41-64 have been added to replace the cancelled claims. Claims 41-64 are pending in this application. Support for the new claims is found in the original claims and the present specification, particularly at Examples 4 and 5; page 6, lines 1-22; and at page 8, lines 8-29. Accordingly, the amendment does not constitute the addition of new matter. In an effort to expedite prosecution of the present application, the original claim language has been modified in view of telephonic interviews with Examiner Leffers on February 5, 2001, April 13, 2001 and April 18, 2001 in related application No. 09/318,288. Applicant respectfully requests the entry of the amendment.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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